appended remarks.

This response will follow the format of the Office Action, page by page.

Page two of the Office Action, third paragraph, noted informalities (grammatical and spelling errors in the specifications). The amendment to the specifications below, substituting entire paragraphs for paragraphs in which there are informalities is believed to overcome these rejections.

In the Specification:

(1) In the specifications, page one, third full paragraph, please replace the paragraph as originally filed with the paragraph as set forth below:

a1 Aldehyde fixation tends to cause substantial changes to the structure of the tissue sample. These changes often tend to cause the antigens that may be present in the tissue samples to lose their reactivity toward antibodies that target such antigens. One effect of formalin fixation is to substantially lock the three dimensional shape of protein molecules within the tissue samples. Because of the recent development of new immunohistochemical reagents, immunohistochemical analyses may now be performed that were impossible to perform at the time many tissues were originally stored. Therefore, a number of procedures have been developed which could reverse some of the changes produced by aldehyde fixation, and enhance the immunohistochemical staining properties of the tissue sample.

(2) In the specifications, page two, please replace the first full paragraph found on page two of the original application with the following:

a2 One method for improving the staining abilities of tissue samples which have been fixed in formalin and embedded in acrylamide gel relates to treatment of acrylamide gel embedded tissue in

1.0% 2-mercaptoethanol for 15 minutes, followed by rinsing with phosphate buffered saline. This treatment allowed the tissue samples to be stained by a number of staining reagents. A method for restoring the immunohistochemical staining properties of tissue samples is described in U.S. Patent No. 5,244, 787 to Key et al. This method involved removing the embedding medium in a pretreatment step. For Paraffin-embedded tissue samples, this pretreatment was accomplished by clearing the tissue samples in xylenes and rehydrating the samples. After the embedding medium has been removed, the sample may be heated in either de-ionized water, an aqueous solution of a zinc salt, or an aqueous solution of a lead salt. The tissue samples were reported to show improved immunohistochemical staining properties when heated in a microwave oven. Improvement was reportedly seen when the solution was heated to its boiling temperature. In general, microwave heating appears to have been found by Key et al to give better results than conventional heating. Solutions containing zinc or lead salts apparently gave significantly better results than de-ionized water.

(3) In the specifications, page three, please replace the second full paragraph on page three of the application as originally filed with the following:

The above mentioned methods inadequately address, among other things, the restoration of paraffin embedded tissue samples in a single reaction step. The procedures described above are usually performed on deparaffinized samples. Typically, the paraffin embedding medium is removed from the samples by successive immersion through a series of xylenes. Following removal of the paraffin embedding medium, the tissue must then be rehydrated by treatment with a series of ethanol-water solutions ranging typically from 100% ethanol to 90% ethanol. Finally, after the sample is rehydrated, the sample must be treated with a solution to reverse the effects of formalin

fixation a step known as unmasking. It is therefore desirable that a single solution be provided that allows the steps of deparaffination (or de-embedding), rehydration, and unmasking of embedded tissue samples to be combined.

(4) In the specifications, on page four, please replace the second full paragraph with the paragraph as set forth below:

In another embodiment of the composition the removing agent includes aqueous solutions of at least one of the following emulsifiers, including detergents and surfactants: Igepal-630 (sigma #3021); Tween 20 (sigma #P7949); Brij 35 (sigma #P1254); Brij 90 (sigma #P1254); Triton X-100 (sigma #T9284); CD TAB (sigma #C5335); and Tween 80 (sigma #P8074). Reference to "sigma" is reference to © 1999 Sigma - Aldrich catalog entitled: "Biochemicals and Reagents for Life Science Research", which is incorporated herein by reference. ~~An aqueous composition is provided for enhancing antigenicity comprising up to about 25% by volume of surfactants.~~

(5) In ~~the~~ specifications, on page six, please replace the first full paragraph with the paragraph as set forth below:

Tissue sections obtained from clinical or animal experimentation frequently are fixed, embedded and stored in a form suitable for later examination by light microscopy. Traditional fixation methods frequently have employed aldehyde fixatives, especially formaldehyde, which preserves the integrity of the tissue samples as well as protects the sample from microbial attack. Tissue antigens tend to be masked, that is, the antigens within the tissue are no longer reactive toward antibodies. When aldehyde based fixatives are used, this masking of the antigens is thought to be due to the reaction of the aldehyde with the tissue proteins. During the fixation process, the aldehyde presumably fixes the tissue by causing cross-linking reactions within and between tissue proteins, as well as causing other unknown changes to the tissue structure. These cross-links within

the tissue proteins tend to alter the three dimensional shape of the protein, preventing access of antibodies to the antigens. After tissue samples have been fixed, they are typically embedded in an embedding medium, such as paraffin or celloidin, so that the samples may be cut into thin sections. The embedding process is preferably accomplished by soaking the tissue samples within the embedding medium such that the tissue samples are substantially surrounded by the embedding medium. In many cases the embedding medium may also soak into the interior of the tissue samples. The embedding medium may prevent the tissue samples from being stained during an immunohistochemical staining procedure.

(6) ~~In the~~ specifications, on page six of the original application, please replace the second paragraph which begins on page six and continues to about half of page seven with the paragraph as set forth below:

Recently, new techniques in immunohistochemical staining of tissue samples have been developed. In general tissue samples are studied for the presence of different types of cells. The specific type of cells being studied may be stained, in the presence of other cells, by the application of immunohistochemical staining techniques. During an immunohistochemical staining process the tissue sample may be reacted with an antibody which specifically binds with the type of cells being studied, and no other type of cells. The bound cell-antibody complex may now be stained, without staining any of the other cells, to allow the stained cells to be easily distinguished from the other cells in the tissue sample. These techniques typically require the antigens to be unmasked before use. Because of the wide spread use of formaldehyde as a fixation chemical or a constituent thereof, it is desirable to provide a procedure whereby the antigenic activity of these tissue samples may be restored. Such a procedure may also take into account the embedding medium of the tissue samples. Formalin-fixed tissue samples are commonly embedded in paraffin before use. Removal of a portion

of the embedding medium is thought to be necessary before immunohistochemical staining may be accomplished.

(7) In the specifications, on page nine, please replace the second full paragraph with the paragraph as set forth below:

The tissue activating agent is preferably adapted to interact with the tissue sample such that the morphology of the components of the tissue are altered. In general the tissue sample contains a number of biological components, including proteins and nucleic acids. Each of these components have a specific three dimensional structure related to the composition of the component. During the fixation, the tissue sample may be treated with an aqueous solution of formaldehyde. The formaldehyde reacts with the components to alter the three dimensional shape of these components, i.e. alter the morphology. These alterations tend to make the tissue samples substantially unreactive toward various immunohistochemical staining protocols. The tissue activating agent is preferably adapted to further alter the morphology of the tissue samples, such that the tissue samples are more reactive toward immunohistochemical reagents. While the tissue activating reagent restores some of the reactivity of the tissue samples, it may not be necessary that the tissue be restored to its original morphology to increase the reactivity of the samples toward immunohistochemical stains.

(8) In the specifications, page eleven, fourth full paragraph (continuing on to top of page twelve), please replace with the paragraph as set forth below:

The composition, as described in previous embodiments, may be used to enhance the immunohistochemical staining ability of formalin-fixed embedded tissue samples. Tissue samples are preferably prepared by soaking the tissue in a buffered formalin or other fixative solution. After soaking for the appropriate time, the tissue sample is preferably dehydrated in ethanol, cleared using xylenes and embedded into paraffin blocks. The embedded tissue is preferably cut into sections up to about 5 micron sections; more preferably into about 3 micron sections. The tissue sample may be mounted onto a positively

charged slide. The tissue sample is preferably mounted onto a poly-L-lysine coated slide. Poly-L-lysine is a positively charged, high molecular weight polymer of the amino acid lysine which, when coated onto a microscope slide, tends to act as a tissue adhesive bonding the tissue to the slide. The use of a tissue adhesive may be necessary to prevent the detachment of the tissue sample from the slide. After the samples have been mounted they may be dried for at least one hour at a temperature of about 58°C.

(9) In the specifications, on page thirteen, please replace the third full paragraph with the paragraph as set forth below:

To the buffered solution prepared as described above was added 18.5 mL of SIMPLE GREEN (Sunshine Makes Inc., Huntington Harbor, California). The pH of the resulting solution was adjusted by addition of concentrated hydrochloric acid or 4N potassium hydroxide as needed to obtain a pH of about 5.96 to 6.04. The resulting tissue enhancing composition may be used to enhance the immunohistochemical staining of tissue samples.

(10) In the specifications, on page fifteen, please replace the first full paragraph with the paragraph as set forth below:

The staining of tissue samples is detailed in the Cell Marque 1998 and 2000 Products and Reference Guide, which are incorporated by reference as if fully set forth herein. Tissue sections on slides (prepared as described above) were treated with a solution of excess antibody for an appropriate amount of time, such time varying depending on the antibody and the protocol being used. The slides were rinsed with an IHC wash buffer. The slides were placed in a Peroxide Block solution for 10 minutes. After this time the slides were rinsed with IHC wash buffer The slides were then placed in a Biotinylated Link solution for 10 minutes at room temperature. After this time the slides were rinsed with IHC wash buffer. The slides were then placed in a Label solution for 10 minutes at room temperature. The Label solution enhances the sensitivity of the antibodies toward the chromogen. After this time the slides were again washed in an IHC wash buffer. The slides were placed in Chromogen solution for 10 minutes at room temperature. After this time the slides

were removed and rinsed with de-ionized water. The slides were then placed in hematoxylin counterstain for 30 seconds at room temperature. The slides were finally rinsed with IHC wash buffer and following an appropriate mounting procedure a coverslip is placed over the tissue samples. All of the above mentioned solutions were obtained from Cell Marque Corp., Austin, Texas.

(11) In the specifications, page eighteen, please replace the second full paragraph beginning on page eighteen and concluding on the top of page nineteen with the paragraph as set forth below:

In addition to the results shown above, additional tests were performed using breast, prostate, thyroid, appendix, brain, lymph, skin, pancreas, colon, muscle bone marrow, and placenta tissue. Staining with these tissue samples also showed that the use of Composition 2 improved staining of these embedded tissue samples without any prior removal of the embedding medium. It should be noted that the samples treated with Composition 1 were deparaffinized prior to treatment, but the samples treated with Composition 2 were <u>not</u> deparaffinized prior to treatment. The use of a composition prepared according to the above embodiments, such as Composition 2, allows this deparaffinization step to be omitted and yet staining of the tissue samples of comparable or even better quality may be obtained.

(12) In the specifications, on page nineteen, please replace the first full paragraph with the paragraph as set forth below:

While the above Exp. #4 was performed on tissue samples that have been heated in the above mentioned compositions, it should be appreciated that the reactions may also be performed at temperatures lower than described in the experiments. The treatment temperature may effect the rate of the tissue enhancing process. The rate of embedding medium removal, enhancement of the tissue sample, and rehydration of the tissue sample may all increase as the temperature is elevated. When the temperature is lowered, e.g. when the reaction is run at room temperature, the rate of these three processes may also be